The non-elected claims are being cancelled and several other claims are also being cancelled in order to reduce the issues.

With entry of this amendment, the claims in the case would be claim 2 amended to be in independent form, claims 3 and 11-15. These claims are directed to the GDF-1 DNA sequence (claims 2 and 3) and use thereof in a construct (claim 11), host cell (claims 12-14) and for expression (claim 15).

The Examiner is requested to reconsider the rejection of the claims under 35 USC 101 as lacking utility. Clearly, if no other use was available, the sequence would be useful as a probe. However, apart from this, the applicant submits that there is patentable utility in the claimed invention. This is shown by recently available evidence showing that the applicant's GDF-1 is useful to inhibit fusion of myoblasts. This is shown by the following:

Innervated tissue, such as skeletal muscle, produce neurotrophic factors that interact with and sustain peripheral nerves. Conversely, factors produced by peripheral nerves may interact with muscle to affect muscle development and maintenance.

Upon the withdrawal of serum, myoblasts can fuse *in vitro* to generate myotubes, which are the cellular components of muscle

fibers. The effect of rhGDF-1 on this fusion process has been investigated.

Preparation of rhGDF-1

Human cDNA encoding GDF-1 was expressed in Chinese hamster ovary (CHO)-DG44 cells using the expression plasmid, pcdhfrpolyA. Roller bottle cultures of a stable recombinant cell line were used to prepare serum-free conditioned media containing rhGDF-1. The rhGDF-1 protein was partially purified by cation exchange chromatography. Conditioned medium from untransfected CHO-DG44 cells was subjected to an identical fractionation by cation exchange chromatography and a fraction corresponding to the same eluant fraction as rhGDF-1 was collected for use as a control. By Coomassie blue staining, the purity of rhGDF-1 was estimated to be in the range of 1-5%.

Myoblast Fusion Assay

Human myoblasts of clonal origin (057A) were grown to confluency in a 24 well plate in medium containing DMEM, 20% FCS, 10 mM HEPES, 4 mM glutamine and penicillin/streptomycin. At confluence, cultures were transferred to fusion medium which was identical to the above except that the serum level was reduced to 0.5%. On assay day 1, the following 3 concentrations of the partially pure rhGDF-1 sample were added to the culture wells:

1:5, 1:10 and 1:100. Correspondingly, control cultures were treated with the CHO-DG44 control sample at the same concentrations. Medium and factor were changed after two days and the experiment was terminated on day 4 by fixing cells in paraformaldehyde. The cultures were then Giemsa stained and myotubes counted under the microscope.

Results

A chart with experimental details, displaying the results of this experiment, is attached hereto. TGF-ß and bFGF are used as positive controls in this experiment. These factors are known to inhibit fusion of myoblasts under the conditions of this experiment. The base line of zero for fusion inhibition is set with just fusion medium alone (no added factors). The addition of the CHO-DG44 control sample did not inhibit fusion of the myoblasts. However, the rhGDF-1 sample produced a significant inhibition of myoblast fusion (up to 83% at the highest dose). The level of inhibition was dose dependent and significant inhibition was still observed at a 1:1000 dilution of the sample. The potency of this rhGDF-1 sample to effect inhibition of fusion is estimated to be at least as high as that of TGF-ß or bFGF.

The indicated inhibition of myoblast fusion using GDF-1 should be more than enough to establish patentable utility for

the presently claimed subject matter. Restricting fusion of muscle myoblasts offers the possibility of treating muscle diseases and disorders that involve hypertrophy (abnormal enlargement) of muscle fibers. Such diseases include spinal muscular atrophy (hypertrophy of type 1 fibers), motor neuron disease (hypertrophy of type 2 fibers), and chronic neuropathy (hypertrophy of type 1 fibers). Moreover, in diseases such as Duchenne muscular dystrophy, where precursor cells become rapidly depleted in the continuous muscle fiber regeneration process, the advanced stages of muscle wasting could be delayed by reducing the rate at which myoblast fusion occurs. Obviously, a great deal of work needs to be done to establish therapeutic utility but the foregoing results should be sufficient to satisfy requirements for a patentable utility.

Reconsideration and withdrawal of the Section 101 rejection are, therefore, requested.

For essentially similar reasons, the Examiner is requested to reconsider the Section 112 objection to the specification and the related rejection of the claims. Those in the art would have no difficulty in obtaining the presently claimed sequence on the basis of the applicant's disclosure and preparing vectors for cell transformation using this sequence, all as disclosed and

claimed. Furthermore, the specification provides more than enough detail to enable one to practice the applicant's method of claim 15.

Attached for the record is a copy of the PCT search report in the PCT filing corresponding to the present case. The Examiner will be familiar with these search results although the references do not seem to have been specifically cited herein. The first and third references are too late to be citable herein and the other two are not believed relevant to the present subject matter as claimed.

Favorable reconsideration is requested.

Respectfully submitted,

CUSHMAN, DARBY & CUSHMAN

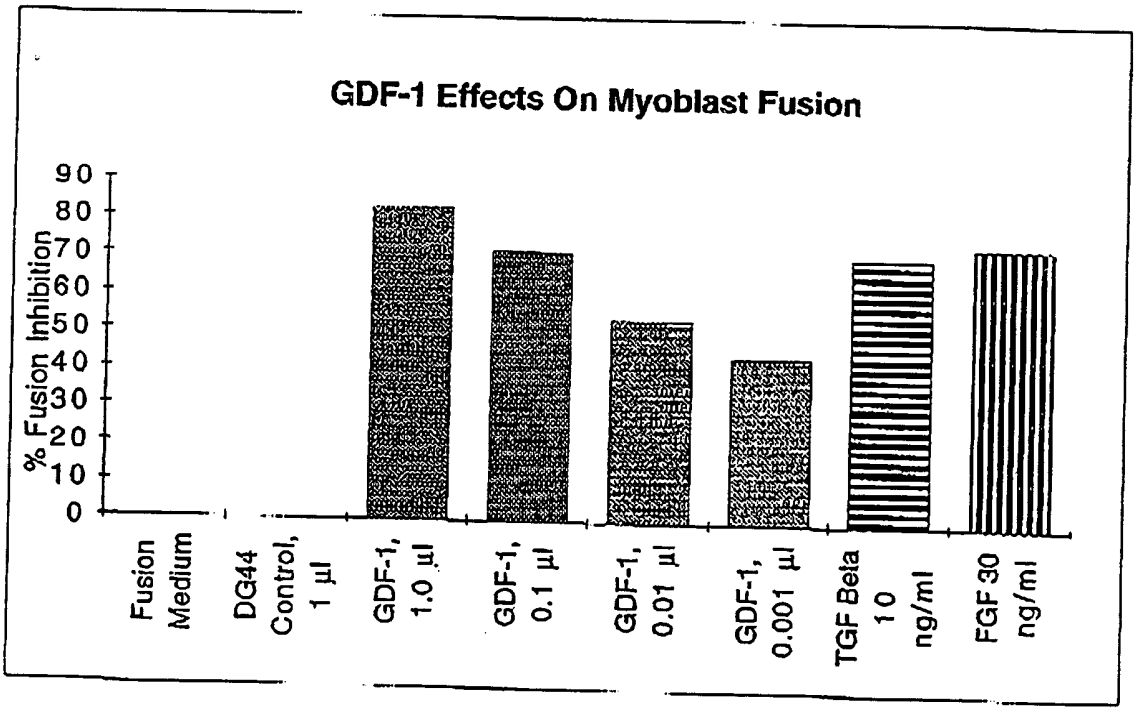
By _____
Paul N. Kokulis
Reg. No. 16773

PNK:mh
1100 New York Avenue, N.W.
Ninth Floor
Washington, D.C. 20005-3918
Phone: (202) 861-3503

GDF 052093

Data Summary	Myotubes/mm^2	STDEV	TTEST
Experiment by Cassandra Kirk			
Fusion Medium	4.94	1.8	
DG44 Control, 1 µl	4.81	2.11	
GDF-1, 1.0 µl	0.83	0.606	0.00129135
GDF-1, 0.1 µl	1.4	0.766	0.00369717
GDF-1, 0.01 µl	2.27	1.08	0.02172506
GDF-1, 0.001 µl	2.75	1.3	0.05879237
TGF Beta 10 ng/ml	1.44	1.21	0.00695176
FGF 30 ng/ml	1.27	0.852	0.00332858
Insulin 200 ng/ml	38.2	2.73	4.7915E-07

	% Inhibition
Fusion Medium	0
DG44 Control, 1 µl	0
GDF-1, 1.0 µl	83
GDF-1, 0.1 µl	72
GDF-1, 0.01 µl	54
GDF-1, 0.001 µl	44
TGF Beta 10 ng/ml	71
FGF 30 ng/ml	74
Insulin 200 ng/ml	-673



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12P 21/00, 21/02, C12N 15/00
C12N 5/00

A1

(11) International Publication Number: WO 92/00382

(43) International Publication Date: 9 January 1992 (09.01.92)

(21) International Application Number: PCT/US91/04096

(22) International Filing Date: 14 June 1991 (14.06.91)

(30) Priority data:
538,372 15 June 1990 (15.06.90) US
614,452 16 November 1990 (16.11.90) US

(71) Applicant: CARNEGIE INSTITUTION OF WASHINGTON [US/US]; 1530 P Street, N.W., Washington, DC 20005 (US).

(72) Inventor: LEE, Se-Jin ; 2509A Steele Road, Baltimore, MD 21209 (US).

(74) Agents: KOKULIS, Paul, N. et al.; Cushman, Darby & Cushman, 1615 L Street, N.W., Eleventh Floor, Washington, DC 20036 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: GDF-1

(57) Abstract

The present invention relates to a DNA segment encoding a mammalian GDF-1 protein and to the protein encoded therein. The invention further relates to a recombinant DNA molecule comprising a nucleotide sequence encoding mammalian GDF-1 protein, and host cells transformed therewith. The invention further relates to a mammalian UOG-1 protein and to a DNA segment encoding same.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland			SN	Senegal
CI	Côte d'Ivoire	KR	Republic of Korea	SU	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
CS	Czechoslovakia	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/04096

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12P 21/00, 21/02; C12N 15/00, 5/00

U.S. Cl: 435/69.4, 69.9, 172.1, 172.3, 240.1, 240.2, 320.1, 252.33; 536/27; 530/350, 399

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.Cl:	435/69.4, 69.9, 172.1, 172.2, 172.3, 240.1, 240.2, 320.1, 252.33; 536/27; 530/350, 399

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

APS, Dialog (files 5, 155, 351, 357 and 358), search terms: uog, gdf, tgf, transforming growth factor beta, superfamily, supergene, DNA, protein

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y, P	THE EMBO JOURNAL, Volume 9, No. 7, issued July 1990, E. Ozkaynak et al., "OP-1 cDNA Encodes an Osteogenic Protein in the TGF-β Family" pages 2085-2093, see entire document.	1-3, 11-14, 16, 19-20
X / Y	US, A, 4,886,747 (DERYNCK ET AL) 12 DECEMBER 1989, see entire document.	1, 3-21
X, P	Proceedings of the National Academy of Sciences, Vol. 88, issued May 1991, Lee, "Expression of growth/ differentiation factor 1 in the nervous system: Conservation of a bicistronic structure", pages 4250-4254, see entire document.	1-21
Y	Proceedings of the National Academy of Sciences, Vol. 86, issued June 1989, Lyons et al., "Vgr-1, a mammalian gene related to Xenopus VG-1, is a member of the transforming growth factor β gene superfamily", pages 4554-4558, see entire document.	1-3, 11-14, 16, 19-20

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 November 1991	10 DEC 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Marianne Porta